# ROLLING CIRCLE REPLICATION REPORTER SYSTEMS

Disclosed are compositions and a method for of amplifying nucleic acid sequences useful for detecting the presence of molecules of interest. The method is useful for detecting specific nucleic acids in a sample with high specificity and sensitivity. The method also has an inherently low level of background signal. A preferred form of the method consists of a DNA ligation operation, an amplification operation, and a detection operation. The DNA ligation operation circularizes a specially designed nucleic acid probe molecule. This operation is dependent on hybridization of the probe to a target sequence and forms circular probe molecules in proportion to the amount of target sequence present in a sample. The amplification operation is rolling circle replication of the circularized probe. A single round of amplification using rolling circle replication results in a large amplification of the circularized probe sequences. Following rolling circle replication, the amplified probe sequences are detected and quantified using any of the conventional detection systems for nucleic acids such as detection of fluorescent labels, enzyme-linked detection systems, antibody-mediated label detection, and detection of radioactive labels. Because, the amplified product is directly proportional to the amount of target sequence present in a sample, quantitative measurements reliably represent the amount of a target sequence in a sample. Major advantages of this method are that the ligation step can be manipulated to obtain allelic discrimination, the DNA replication step is isothermal, and signals are strictly quantitative because the amplification reaction is linear and is catalyzed by a highly processive enzyme. In multiplex assays, the primer oligonucleotide used for the DNA polymerase reaction can be the same for all probes. Also described are modes of the method in which additional amplification is obtained using a cascade of strand displacement reactions.